

WHAT IS CLAIMED IS:

1. A composition for use in reverse transcription of a nucleic acid molecule, said composition comprising two or more polypeptides having reverse transcriptase activity.
- 5 2. The composition of claim 1, wherein said polypeptides are obtained from different sources.
3. The composition of claim 1, wherein the transcription pause site of each of said polypeptides is different from that of each of the other polypeptides in said composition.
- 10 4. The composition of claim 1, wherein said polypeptides are reduced or substantially reduced in RNase H activity.
- 15 5. The composition of claim 4, wherein said polypeptides are selected from the group consisting of M-MLV H⁻ reverse transcriptase, RSV H⁻ reverse transcriptase, AMV H⁻ reverse transcriptase, RAV H⁻ reverse transcriptase, MAV H⁻ reverse transcriptase and HIV H⁻ reverse transcriptase, and derivatives, variants, fragments or mutants thereof.
- 20 6. The composition of claim 5, wherein said AMV H⁻ reverse transcriptase is selected from the group consisting of AMV α H⁻/βH⁻ reverse transcriptase, AMV α H⁻/βH⁺ reverse transcriptase, AMV βH⁻/βH⁻ reverse transcriptase, AMV βH⁺/βH⁻ reverse transcriptase, AMV βp4/βp4 reverse transcriptase, and AMV α H⁻ reverse transcriptase, and derivatives, variants, fragments or mutants thereof.

7. The composition of claim 5, wherein said RSV H⁻ reverse transcriptase is selected from the group consisting of RSV α H⁻/ β H⁻ reverse transcriptase, RSV α H⁻/ β H⁺ reverse transcriptase, RSV β H⁻/ β H⁻ reverse transcriptase, RSV β H⁺/ β H⁻ reverse transcriptase, RSV β p4/ β p4 reverse transcriptase, and RSV α H⁻ reverse transcriptase, and derivatives, variants, fragments or mutants thereof.

8. The composition of claim 1, wherein said polypeptides are selected from the group consisting of *Taq*, *Tne*, *Tma*, *Pfu*, VENTTM, DEEPVENTTM and *Tth* DNA polymerases, and mutants, fragments, variants and derivatives thereof.

9. The composition of claim 1, wherein said polypeptides are present in said composition at working concentrations.

10. A method for reverse transcription of one or more nucleic acid molecules comprising

(a) mixing one or more nucleic acid templates with two or more polypeptides having reverse transcriptase activity; and

(b) incubating said mixture under conditions sufficient to make one or more first nucleic acid molecules complementary to all or a portion of said one or more templates.

11. The method of claim 10, wherein said nucleic acid template is a messenger RNA molecule or a population of mRNA molecules.

12. The method of claim 10, said method further comprising incubating said one or more first nucleic acid molecules under conditions sufficient to make one or more second nucleic acid molecules complementary to all or a portion of said one or more first nucleic acid molecules.

13. A cDNA molecule made according to the method of claim 10.

14. A cDNA molecule made according to the method of claim 12.

15. A method for amplifying one or more nucleic acid molecules, said method comprising

(a) mixing one or more nucleic acid templates with two or more polypeptides having reverse transcriptase activity and one or more DNA polymerases; and

(b) incubating said mixture under conditions sufficient to amplify one or more nucleic acid molecules complementary to all or a portion of said one or more templates.

16. A method for amplifying one or more nucleic acid molecules, said method comprising

(a) mixing one or more nucleic acid templates with two or more polypeptides having reverse transcriptase activity and DNA polymerase activity; and

(b) incubating said mixture under conditions sufficient to amplify one or more nucleic acid molecules complementary to all or a portion of said one or more nucleic acid templates.

17. A nucleic acid molecule amplified according to the method of claim 15 or claim 16.

18. A vector comprising the cDNA molecule of claim 14.

19. The vector of claim 18 wherein said vector is an expression vector.

a

20. A host cell comprising the cDNA molecule of claim 14.

21. A method for sequencing one or more nucleic acid molecules, said method comprising

(a) mixing one or more nucleic acid molecules to be sequenced with one or more primers, two or more polypeptides having reverse transcriptase activity, one or more nucleotides and one or more terminating agents;

(b) incubating said mixture under conditions sufficient to synthesize a population of molecules complementary to all or a portion of said one or more molecules to be sequenced; and

(c) separating said population to determine the nucleotide sequence of all or a portion of said one or more molecules to be sequenced.

22. A kit for use in reverse transcription, amplification or sequencing of a nucleic acid molecule, said kit comprising two or more polypeptides having reverse transcriptase activity.

23. The kit of claim 22, said kit further comprising one or more components selected from the group consisting of one or more nucleotides, one or more DNA polymerases, a suitable buffer, one or more primers and one or more terminating agents.

24. The kit of claim 23, wherein said terminating agent is a dideoxynucleotide.

25. The kit of claim 23, wherein two or more of the components of said kit are present as a mixture or are present as separate components.

26. A method of producing an ASLV reverse transcriptase, said method comprising

- 5 (a) obtaining a host cell comprising one or more nucleic acid sequences encoding one or more subunits of ASLV reverse transcriptase; and
- (b) culturing said host cell under conditions sufficient to produce said ASLV reverse transcriptase subunits.

27. The method of claim 26, wherein said one or more nucleic acid sequences encoding one or more subunits of ASLV reverse transcriptase are contained in one or more vectors.

10 28. The method of claim 26, wherein said ASLV reverse transcriptase subunits are selected from the group consisting of one or more α subunits, one or more β subunits, and one or more $\beta p4$ subunits, of one or more ASLV reverse transcriptases, and derivatives, variants, fragments or mutants thereof.

15 29. The method of claim 26, wherein said subunits are one or more α subunits.

30. The method of claim 26, wherein said subunits are one or more β subunits.

31. The method of claim 26, wherein said subunits are one or more $\beta p4$ subunits.

20 32. The method of claim 26, wherein said subunits are one α subunit and one β subunit of one or more ASLV reverse transcriptases.

a

33. The method of claim 26, wherein said subunits are co-expressed to form an ASLV reverse transcriptase and wherein said ASLV reverse transcriptase is isolated from said host cell.

5 34. The method of claim 26, wherein said subunits of ASLV reverse transcriptase are isolated and then mixed to form an ASLV reverse transcriptase.

35. The method of claim 30, wherein said β subunits form an ASLV reverse transcriptase comprising two β subunits.

36. The method of claim 32, wherein said α and β subunits form an ASLV reverse transcriptase comprising an α and a β subunit.

10 37. The method of claim 26, wherein one or more of said subunits have been modified to reduce or substantially reduce the RNase H activity in said subunits.

38. The method of claim 26, wherein said subunits are encoded by one or more nucleotide sequences contained on the same or on different vectors.

15 39. The method of claim 26, wherein said ASLV reverse transcriptase is an RSV reverse transcriptase.

40. The method of claim 26, wherein said ASLV reverse transcriptase is an AMV reverse transcriptase. *a*

20 *Sub C3* 41. An ASLV reverse transcriptase produced according to the method of claim 26. */a*

42. The ASLV reverse transcriptase of claim 41, wherein said ASLV reverse transcriptase is selected from the group consisting of an ASLV $\alpha\beta$ reverse transcriptase, an ASLV $\beta\beta$ reverse transcriptase, an ASLV $\beta p4\beta p4$ reverse transcriptase, and an ASLV α reverse transcriptase.

5 43. The ASLV reverse transcriptase of claim 41, wherein said ASLV reverse transcriptase is reduced or substantially reduced in RNase H activity.

44. The ASLV reverse transcriptase of claim 41, wherein said ASLV reverse transcriptase is an RSV reverse transcriptase.

10 45. The ASLV reverse transcriptase of claim 41, wherein said ASLV reverse transcriptase is an AMV reverse transcriptase.

46. An isolated nucleic acid molecule comprising a nucleotide sequence encoding one or more subunits of ASLV reverse transcriptase.

15 47. The nucleic acid molecule of claim 46, wherein said molecule encodes one or more α subunits of ASLV reverse transcriptase, or a derivative, fragment or mutant thereof.

48. The nucleic acid molecule of claim 46, wherein said molecule encodes one or more β subunits of ASLV reverse transcriptase, or a derivative, fragment or mutant thereof.

20 49. The nucleic acid molecule of claim 46, wherein said molecule encodes one or more $\beta p4$ subunits of ASLV reverse transcriptase, or a derivative, fragment or mutant thereof.

50. The nucleic acid molecule of claim 46, wherein said molecule encodes α and β ASLV reverse transcriptase subunits, or derivatives, variants, fragments or mutants thereof.

51. A vector comprising the nucleic acid molecule of claim 46.

52. A host cell comprising the nucleic acid molecule of claim 46.

53. The vector of claim 51, wherein said vector is plasmid pDABH-His.

54. The host cell of claim 52, wherein said host cell is *E. coli* DH10B(pDABH-His).

55. The isolated nucleic acid molecule of claim 46, wherein said ASLV reverse transcriptase is an RSV reverse transcriptase.

56. The isolated nucleic acid molecule of claim 46, wherein said ASLV reverse transcriptase is an AMV reverse transcriptase.

57. A method for producing one or more cDNA molecules by reverse transcription of one or more nucleic acid templates comprising

(a) mixing one or more nucleic acid templates with a ASLV RT comprising one or more subunits; and

(b) incubating said mixture under conditions sufficient to make one or more first nucleic acid molecules complementary to all or a portion of said one or more templates.

a

58. The method of claim 57, wherein said subunits are one or more α subunits, one or more β subunits, one or more $\beta p4$ subunits, or a combination thereof.

5 59. The method of claim 57, wherein said ASLV reverse transcriptase is selected from the group consisting of an ASLV $\alpha\beta$ reverse transcriptase, an ASLV $\beta\beta$ reverse transcriptase, an ASLV $\beta p4\beta p4$ reverse transcriptase, and an ASLV α reverse transcriptase.

60. The method of claim 57, wherein said nucleic acid template is a mRNA molecule or a population of mRNA molecules.

10 61. The method of claim 57, wherein said method further comprises incubating said one or more first nucleic acid molecules under conditions sufficient to make one or more second nucleic acid molecules complementary to all or a portion of said one or more first nucleic acid molecules.

15 62. The method of claim 61, wherein said first and said second nucleic acid molecules form a double stranded DNA molecule.

63. The method of claim 62, wherein said double stranded DNA molecule is a full-length cDNA molecule.

64. A cDNA molecule made according to the method of claim 57.

65. A cDNA molecule made according to the method of claim 61.

20 66. A vector comprising the cDNA molecule of claim 65.

a

67. The vector of claim 66, wherein said vector is an expression vector.

68. A host cell comprising the cDNA molecule of claim 65.

69. The method of claim 61, wherein said ASLV reverse transcriptase is an RSV reverse transcriptase.

5 70. The method of claim 61, wherein said ASLV reverse transcriptase is an AMV reverse transcriptase.

71. A method for amplifying one or more nucleic acid molecules comprising

10 (a) mixing one or more nucleic acid templates with one or more ASLV RTs comprising one or more subunits and optionally with one or more DNA polymerases; and

(b) incubating said mixture under conditions sufficient to amplify one or more nucleic acid molecules complementary to all or a portion of said one or more templates.

15 72. The method of claim 71, wherein said subunits are one or more α subunits, one or more β subunits, one or more $\beta p4$ subunits, or a combination thereof.

20 73. The method of claim 71, wherein said ASLV reverse transcriptase is selected from the group consisting of an ASLV $\alpha\beta$ reverse transcriptase, an ASLV $\beta\beta$ reverse transcriptase, an ASLV $\beta p4\beta p4$ reverse transcriptase, and an ASLV α reverse transcriptase.

a

74. The method of claim 71, wherein said ASLV reverse transcriptase is an RSV reverse transcriptase.

75. The method of claim 71, wherein said ASLV reverse transcriptase is an AMV reverse transcriptase.

5 76. A method for sequencing one or more nucleic acid molecules comprising

(a) mixing one or more nucleic acid molecules to be sequenced with one or more primers, an ASLV RT comprising one or more subunits, one or more nucleotides and one or more terminating agents;

10 (b) incubating said mixture under conditions sufficient to synthesize a population of nucleic acid molecules complementary to all or a portion of said one or more nucleic acid molecules to be sequenced; and

15 (c) separating said population of nucleic acid molecules to determine the nucleotide sequence of all or a portion of said one or more nucleic acid molecules- to be sequenced.

77. The method of claim 76, wherein said subunits are one or more α subunits, one or more β subunits, one or more $\beta p4$ subunits, or a combination thereof.

20 78. The method of claim 76, wherein said ASLV reverse transcriptase is selected from the group consisting of an ASLV $\alpha\beta$ reverse transcriptase, an ASLV $\beta\beta$ reverse transcriptase, an ASLV $\beta p4\beta p4$ reverse transcriptase, and an ASLV α reverse transcriptase.

79. The method of claim 76, wherein said ASLV reverse transcriptase is an RSV reverse transcriptase. *a*

80. The method of claim 76, wherein said ASLV reverse transcriptase is an AMV reverse transcriptase. *1a*

81. A kit comprising one or more ASLV RT subunits, or one or more derivatives, variants, fragments or mutants thereof.

5 82. The kit of claim 81, wherein said ASLV RT subunits are one or more ASLV RT α subunits, one or more ASLV RT β subunits, one or more ASLV RT β p4 subunits, or a combination thereof.

83. The kit of claim 81 wherein said ASLV reverse transcriptase is an RSV reverse transcriptase. *B*

84. The kit of claim 81, wherein said ASLV reverse transcriptase is an AMV reverse transcriptase.

85. A kit comprising an ASLV RT, wherein said ASLV RT is selected from the group consisting of an ASLV $\alpha\beta$ RT, an ASLV $\beta\beta$ RT, an ASLV β p4 β p4 RT, and an ASLV α RT, or a derivative, fragment or mutant thereof.

*Sub
a₄* 86. The kit of claim 85, wherein said ASLV RT comprises an α and a β subunit. *1a*

87. The kit of claim 85, wherein said ASLV reverse transcriptase is an RSV reverse transcriptase.

20 88. The kit of claim 85, wherein said ASLV reverse transcriptase is an AMV reverse transcriptase. *B*

89. A method for producing one or more cDNA molecules by reverse transcription of one or more nucleic acid templates comprising

(a) mixing one or more nucleic acid templates with one or more polypeptides having reverse transcriptase activity; and

(b) incubating said mixture at a temperature of about 50°C or greater and under conditions sufficient to make one or more first nucleic acid molecules complementary to all or a portion of said one or more templates.

90. The method of claim 89, wherein said temperature is 60°C or greater.

91. The method of claim 89, wherein said temperature ranges from about 50°C to about 70°C.

92. The method of claim 89, wherein said temperature ranges from about 55°C to about 65°C.

93. The method of claim 89, wherein said nucleic acid template is an RNA or DNA molecule.

94. The method of claim 93, wherein said RNA molecule is an mRNA molecule or a polyA⁺ RNA molecule.

95. The method of claim 89, wherein said nucleic acid template is a population of mRNA molecules.

96. The method of claim 94, wherein said first nucleic acid molecule is a full length cDNA molecule.

a

97. The method of claim 89, further comprising incubating said one or more first nucleic acid molecules under conditions sufficient to make one or more second nucleic acid molecules complementary to all or portion of said one or more first nucleic acid molecules.

5 98. The method of claim 97, wherein said first and said second nucleic acid molecules are DNA molecules.

99. The method of claim 98, wherein said first and said second DNA molecules form a double stranded DNA molecule.

100. The method of claim 99, wherein said double stranded DNA molecule is a full length cDNA molecule.

101. The method of claim 89, wherein said one or more polypeptides having reverse transcriptase activity are reduced or substantially reduced in RNase H activity.

102. The method of claim 89, wherein said one or more polypeptides having reverse transcriptase activity is one or more ASLV reverse transcriptases comprising one or more subunits.

103. The method of claim 102, wherein said one or more ASLV reverse transcriptases is one or more RSV reverse transcriptases.

104. The method of claim 102, wherein said one or more ASLV reverse transcriptases is one or more AMV reverse transcriptases.

105. The method of claim 102, wherein said ASLV RT subunits are one or more ASLV RT α subunits, one or more ASLV RT β subunits, one or more ASLV RT β p4 subunits, or a combination thereof.

5 106. The method of claim 102, wherein said ASLV RT is selected from the group consisting of an ASLV $\alpha\beta$ RT, an ASLV $\beta\beta$ RT, an ASLV β p4 β p4 RT, and an ASLV α RT, and derivatives, variants, fragments or mutants thereof.

107. The method of claim 102, wherein said ASLV RT is ASLV $\alpha\beta$ RT, or a derivative, variant, fragment or mutant thereof.

10 108. The method of claim 102, wherein said one or more subunits are reduced or substantially reduced in RNase H activity.

109. A nucleic acid molecule produced according to the method of claim 89.

110. A nucleic acid molecule produced according to the method of claim 97.

15 111. The nucleic acid molecule of claim 110, wherein said nucleic acid molecule is a full-length cDNA molecule.

112. A vector comprising the nucleic acid molecule of claim 110.

113. The vector of claim 112, wherein said vector is an expression vector.

20 114. A host cell comprising the nucleic acid molecule of claim 109.

/a

115. A host cell comprising the nucleic acid molecule of claim 110.

1a

add B1

add B1

add B1